Department

Subject

Name IN KIM #7

Address 102894

National Brand

43-648

Computation Notebook

Dennison Stationery Products Co., Framingham, MA 01701

75 Sheets
11¾" x 9¼"
4x4 Quad.

0 73333 43648 8

Ruben EXHIBIT #90

Department

Subject

Name [redacted] Kim #7

Address [redacted] 102894

National Brand

43-648

Computation Notebook

Dennison Stationery Products Co., Framingham, MA 01701

75 Sheets
11¾" x 9¼"
4x4 Quad.

0 73333 43648 8

Ruben EXHIBIT 2090
Ruben v. Wiley et al.
Interference No. 105,077
RX 2090

TNT - HE9MF73/HTPAN/HTTACI56/HPODM93 33

8/25/94

TNT on

1. HE9MF73505 — 0.25 ug/ml
2. HE9MF73507 — 0.25 ug/ml
3. HTPAN08504 — 0.25 ug/ml
4. HPODM93 — 0.25 ug/ml
5. HTTCI56 — 0.1 ug/ml

	1	2	3	4	5
Rabbit lysate	25	25	25	25	25
Buffer	2	2	2	2	2
Pol T_3	1	1	1	1	1
AA mix ⊖ Met	1	1	1	1	1
^{35}S meth.	1	1	1	1	1
RNasin	1	1	1	1	1
DNA	4	4	4	4	10
H_2O	15	15	15	15	9
	50	50	50	50	50

Incubate 30°C 1½ hrs.

5ul of TNT + 20ul 1x SDS Buffer.
Heat 95°C 2 min
Chill on ice
Quick Spin.
Run 10 µl on 12.5% SDS Gel
with Rainbow marker.
Fix Gel 20% methanol 10% Acetic Acid
30 min at 37°C
Amplify 30 min 37°C.
Dry gel 70°C ~1½ hrs.

TNT - HE9MF73/HTPAN/HTACI56/HPODM93 33

8/25/94

Do TNT on

① HE9MF73505 0.25 ug/ml
② HE9MF73507 0.25 ug/ml
③ HTPAN08504 0.25 ug/ml
④ HPODM93 0.25 ug/ml
⑤ HTICI56 0.1 ug/ml

	①	②	③	④	⑤
Rabbit lysate	25	25	25	25	25
Buffer	2	2	2	2	2
Pol T_3	1	1	1	1	1
TA mix ⊖ Met	1	1	1	1	1
35S mix	1	1	1	1	1
RA asin	1	1	1	1	1
DNA	4	4	4	4	1
H_2O	15	15	15	15	9
	50	50	50	50	50

Incubate 30°C 1 1/2 hrs

5ul of TNT + 20ul 1X SDS Buffer
Heat 95°C 2 min
Chill on ice
Quick Spin
Run Total on 12.5% SDS Gel
with Rainbow marker CH
Fix Gel 20% methanol 10% Acetic Acid
30 min at 37°C
Amplify 30 min 37°C
Dry gel 70°C @ 1/2 hrs

TNT - HE9MF73/HTPAN/HTTACI56/HPDDM93

8/25/94

Do TNT on.

①	HE9MF73505	0.25 ug/ul
②	HE9MF73507	0.25 ug/ul
③	HTPAV08504	0.25 ug/ul
④	HPDDM93	0.25 ug/ul
⑤	HTTCI56	0.1 ug/ul

pg 149 books 100 DNA#6

	①	②	③	④	⑤
Rabbit Lysate	25	25	25	25	25
Buffer	2	2	2	2	2
Pol T3	1	1	1	1	1
AA mix ⊖ Met	1	1	1	1	1
35S Met.	1	1	1	1	1
RNasin	1	1	1	1	1
DNA	4	4	4	4	10
H2O	15	15	15	15	9
	50	50	50	50	50

Incubate 30°C 1½ hrs

5ul of TNT + 20ul 1X SDS Buffer
Heat 95°C 2 min
Chill on ice
Quick spin
Run 10ul on 12.5% SDS Gel
with Rainbow marker CH

Fix Gel 20% Methanol 10% Acetic Acid
30 min at 37°C
Amplify 30 min 37°C
Dry gel 70°C a 1½ hrs

34 TNT

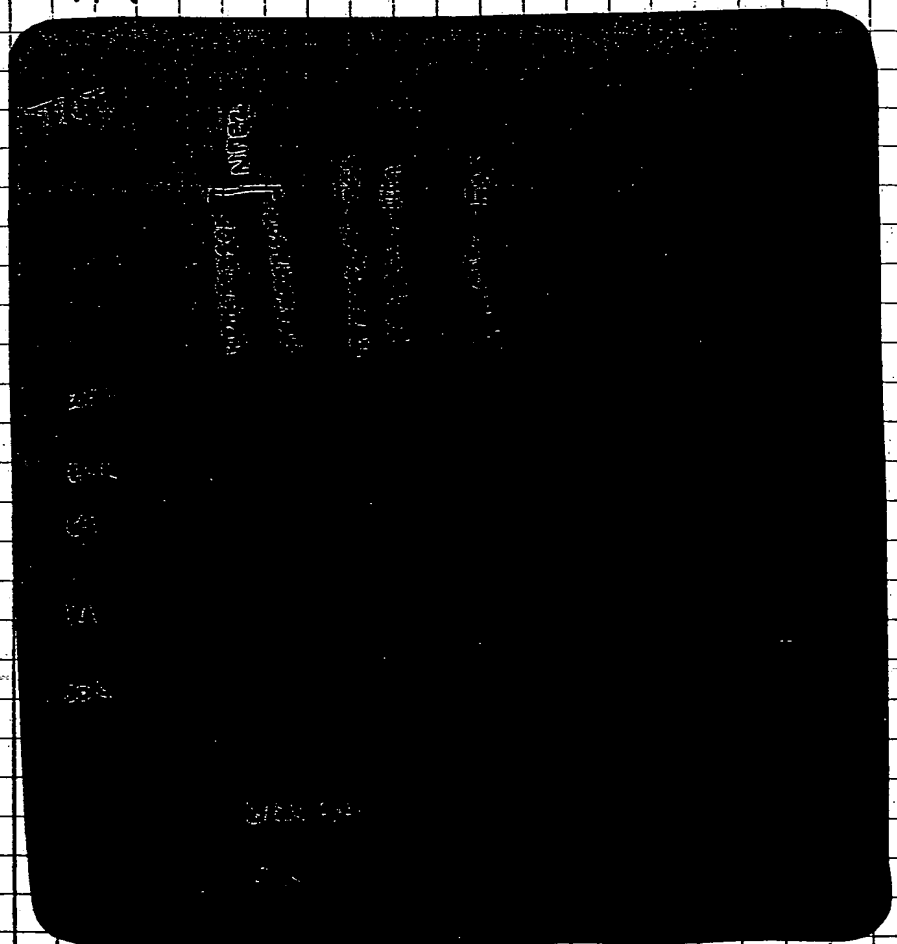
8/25/94

Expose in Small Cassette
-80C O/N.

8/26/94

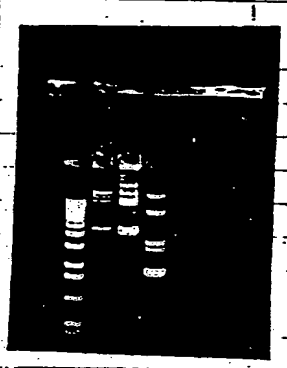
Develop film

	Expected Size
HE9MF73S05	> ~ 83kd
HE9MF73S07	
HTPAN08S04	~ 30.9 kd
HPDDM93	~ 82.4 kd.
HTTC756	~ 30.9 kd



Mini Preps / Maxi Prep

8/18/94



Sequence F & Rev.

Inoculate for maxis
HTTCIS6B
HTPB411C
HMNAD13C
HCTBA03A
HMSB243A
HOSDK13A
} 200 ml LB + Amp

Incubate 37°C O/N.

8/19/94

Qiagen Maxi prep.

Spin Cells 4K 10min
Remove supernatant
Resuspend pellet 10ml P1
Add 10ml P2 Mix
Incubate RT for ~~10~~ 5min.
Add 10 ml Chilled P3
Mix and incubate 20 min on ice
Spin 9K 30min
Equilibrate Tip 500 with 10ml QBT
Apply supernatant through Kimwipe
Wash 2x 30ml QC
Elute 15 ml QF

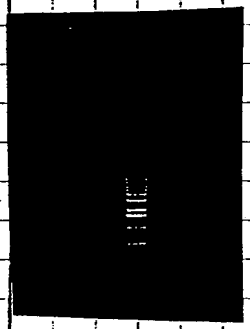
Steven M. Rule
8/19/94

44 HE20742 (fragment pup HTPAN08504 HPRCC91)

8/31/94

Transfer Supernatant to fresh tube.

Run 1/ul on gel with 1Kb Ladder.



HPRCC91 is Wrong!
Trg digesting with Xho I / R I

HE20742 X3/R3 looks good - ~600ng/ul
use to probe filters

Digest HPRCC91 Boiling minis - A & B

DNA	20 ul
10x #2	5
Eco RI	1
Xho I	1
H_2O	23
	50 ul

Incubate 37°C.

Digest HTPAN08504 (Fas Ligand full length)

DNA (0.6 ug/ul)	15 ul
10x #2	10 ul
Eco RI	1 ul
Xho I	1 ul
H_2O	73 ul
	100 ul

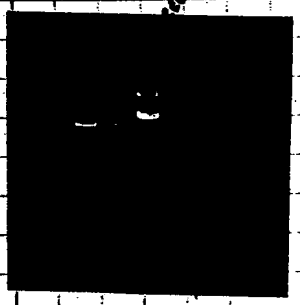
Incubate 37°C

HE2 Continued
pg 47

46 Fragment prep HTPAN08S04 / HPRCC91

8/31/94 (HTPAN08-P534)

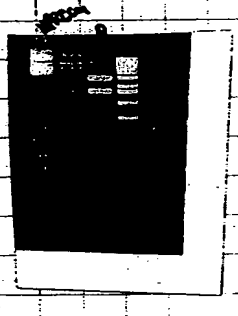
Run 2ul of digest on gel



Does not look like we have Complete Digestion.
Continue to digest O/N at 37°C.

9/1/94

Check Digests
Run 2ul on 1% gel with 1Kb ladder.



HTPAN08S04 looks like it is almost fully Digested.
HPRCC91 does not look digested at all.
Clean up DNA and try again.

2X phenol extract w/ equal volume
2X chloroform extract w/ equal volume
Ethanol / 3M NaAcetate precipitate DNA
1X 70% Ethanol wash
Dry pellet
Resuspend in 50µl TE

Set up Digest

DNA	20µl
10X #2	5µl
H_2O	23µl
Xho	1µl
EcoR1	1µl
	50µl

Incubate at 37°C.

HTPAN08S04 – Protein fragment Prep.

9/2/94

PCR –

HTPAN08S04 1x/rxn.

		12x	
7690 3' Asp 718	0.3	3.6	
7689 5' BamHI	0.3	3.6	
10x PCR	3.5	42	Stds
10 x dNTP	3.5	42	Should
Taq	0.2	2.4	get a
H2O	26.2	314	fragment
DNA	1	12	
	35µl	420	~ 850 bp.

PCR program # 69

95°C 5min
95°C 20sec
55°C 20sec } 30x
72°C 1min
72°C 7½min
4°C Hold

Run 5µl on gel.

[redacted]

looks good –

Add equal Volume of 13% PEG / 1.6M NaCl
Let sit on ice 10 min

Spin 10 min
Remove Supernatant
Add 1ml 70% Ethanol
Spin 5min
Remove Supernatant
Allow Pellet to dry
Resuspend in 100µl TE
Store -20°C

58 HTPAN085O4 - Fragment Prep

9/16/94

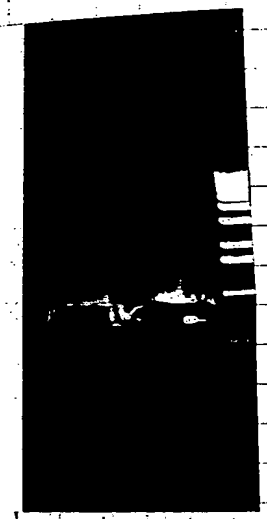
Digest fragment - Asp 718 + BamHI
Asp Buffer B, Bam - Bam Buffer

DNA (PCR product)	20 ul
10x Buffer	5 ul
H2O	24 ul
Enzyme	1 ul
	50 ul

Incubate 37°C 4 hrs.

Add Complement Enzyme.
Incubate 37°C 4 hrs.

Run on 0.8% LMP Agarose gel
with 1Kb ladder.



Cut out fragments into
1.5ml Tube

Add 400 ul NaI
Heat 55°C 5min
Mix
Add 5 ul Glass milk
Vortex
Let sit at RT 5min
Spin 5sec
Remove supernatant
3x { Resuspend pellet in 200 ul
Wash Solution
Spin 5sec
Remove Supernatant }
Spin 5sec
Remove Supernatant

Add 20 ul TE
Heat 55°C 1min
Spin 5sec

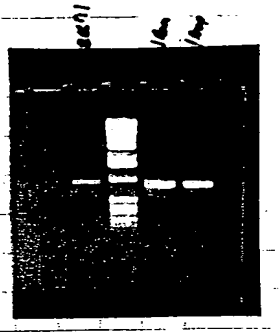
Pg 69

68 FRAGMENT Prep HPRECQL / HTPAN08SQ4 Asp / Bam.

pg66 pg69

9/7/94

Run 1ul on gel with 1Kb Ladder



HPRECQ1 Kpn / Bam HI
~100 ng/ul

HTPAN08SQ4 3' Asp / 5' Bam
Asp / Bam Digest
Gene Cleaned 9/6
~300 ng/ul

HTPAN08SQ4 3' Asp / 5' Bam
Bam / Asp Digest
Gene Cleaned 9/6
~300 ng/ul

Store in Fragment Box -20°C.

HTPAN08SO4 - PAZ Protein Prep

pg 58

9/16/94

Transfer Supernatant to fresh Tube.
Resuspend pellet in 10ul TE
Heat 55°C 1 min
Spin 5 sec
Transfer Supernatant to fresh tube.

Set up ligations using PAZ Asp/Bam digest - (See pg 67)

①		②		③	
HTPAN08SO4 Asp/Bam	3	HTPAN08SO4 A/B	3	HTPAN08SO4 B/A	3
pAZ Asp/Bam	1	PAZ B/A	1	pAZ A/B	1
10X Buffer	1		1		1
H_2O	14		14		14
T4 ligase	1		1		1
	20ul		20		20

④		⑤		⑥	
HTPAN08SO4 B/A	3	HTPAN08SO4 A/B	3	HTPAN08SO4 B/A	3
pAZ B/A	1				
10X Buffer	1		1		1
H_2O	14		15		15
T4 ligase	1		1		1
	20		20		20

⑦		⑧		⑨	
Fragment					
pAZ A/B	1	pAZ B/A	1		
10X Buffer	1		1		1
H_2O	17		17		18
T4 ligase	1		1		1
	20		20		20ul

Incubate 16°C O/N

See pg 68 for fragment Conc

70 HTPAN08S04 Protein. pA2

9/7/94

Thaw DH5α Chemically Competent Cells on ice.

Aliquot 100ul of Cells into fresh tubes
Add 10ul of Ligation Rxn.
Mix gently
Incubate on ice 1hr.
Heat 42°C 45sec
place on ice
Add 400ul LB
Incubate 37°C 1hr.
Plate onto LB + Amp Plates.

① HTPAN08S04 Asp/Bam + pA2 Asp/Bam – 50 & 200ul
② HTPAN08S04 Asp/Bam + pA2 Bam/Asp – 50 & 200ul.
③ HTPAN08S04 Bam/Asp + pA2 Asp/Bam – 100ul
④ HTPAN08S04 Bam/Asp + pA2 Bam/Asp – 100ul
⑤ HTPAN08S04 Asp/Bam – 200ul
⑥ HTPAN08S04 Bam/Asp – 200ul.
⑦ pA2 Asp/Bam – 200ul
⑧ pA2 Bam/Asp – 200ul
⑨ Lig Rxn only – 200ul
⑩ 5ng pA2 plasmid DNA – 200ul
⑪ DH5α Cells only – 200ul.

Incubate 37°C O/N.

9/8/94

Inoculate Colonies into LB+Amp in 96 Well dish
Incubate at 37°C w/aeration 4hrs
PCR to check inserts

HTPAN08SOY Protein pAZ

9/8/94

PCR

		(22x)
① #7089	1	22
#7090	1	22
10xdNTP	3.2	70.4
10x PCR	3.2	70.4
H_2O	21.4	470.8
Taq	0.2	4.4
Culture	2	—
	~~32~~ 32 ul	

PCR Program #69.

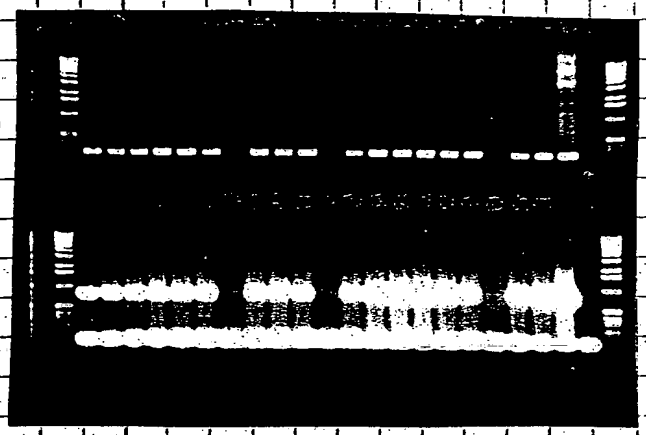
95°C 5min
95°C 20sec
55°C 20sec } 30x
72°C 1min
72°C 7½ min
4°C Hold.

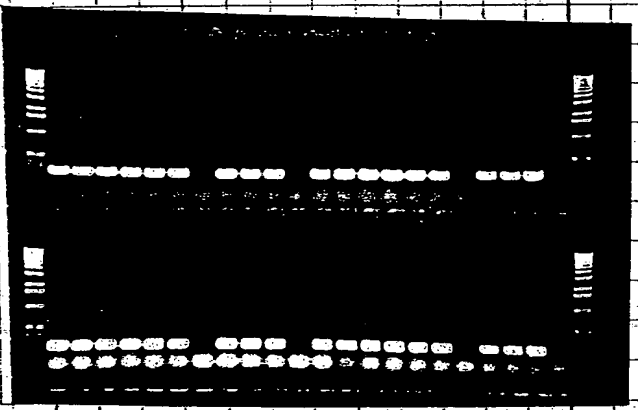
① control pAZ Plasmid DNA 10ng

② control PCR HTPAN08SOY Asp/Bam or fragment

PCR
② HTPAN08RP05 0.2
HTPAN08FP18 0.2
Bam
③ HTPAN08FP16 #7089
④ HTPAN08RP01 #7090

Run 10ul on gel with 1Kb ladder.





There are clearly Positives.
Do boiling mini ① + Sequence with internal primers out through ligation Junction

72 HTPAN08S04 into pAZ

9/8/94

Inoculate 5 ml TB + Amp with all the ⊕ clones -

Incubate 37°C O/N w/aeration

9/9/94

Boiling mini preps

Spin 2 ml of culture in 2 ml Tubes
6 min
Remove supernatant
Resuspend pellet in 450 ul of
STET + RNase + Lysozyme
Boil 1 min
Spin 10 min
Remove pellet w/ toothpick
Add 75 ul of 13% PEG-8000/1.6M NaCl
Vortex well
Spin 10 min
Remove supernatant
Add 1000 ul 70% Ethanol
Mix
Spin 5 min
Remove supernatant
Allow Pellets to dry
Resuspend in 150 ul of TE.
Run 2 ul on gel with 1 kb ladder
and pAZ (Esorg)

B01 → B07

Add 150 ul more TE

Digest to make sure
insert "pops" out.

[redacted]

pg 81

HTPAN08S04 - pA2 Poore. Virus. 81

pg72

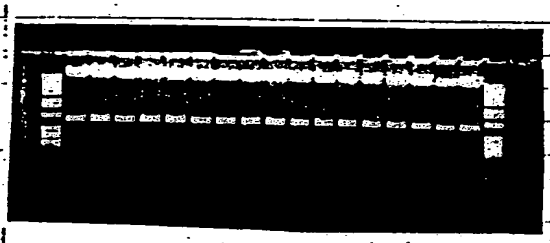
9/9/99

HTPAN08B01 - 17
Digest with Bam/Asp.

		(17x)
DNA	5 µl	
10x B	3	51
H_2O	21.6	367.2
Bam/Asp	0.2 / 0.2	3.4 / 3.4
	30 µl.	

Incubate 37°C 2 hrs.

Run 10 µl on gel with 1kb ladder



They all look correct.

Submit for Sequencing to confirm Sequence and to confirm Cloning sites are OK

Submit in 96 Well dish

HTPAN08S04
HTPAN08B01
B02
B03
B04
B05

Use Primers: RP01 RP12
FP03 FP14
RP05 FP16
RP06 FP17
RP07 FP18

freeze Glycerols of B01 - B17 -

82 HTPAN08504 - Baculovirus FAS Maxi Prep

9/12/94

Inoculate 150 ml TB + Amp
with glycerol stock of
HTPAN08B01
HTPAN08B02
Incubate 37°C O/N w/ aeration

9/13/94

Qiagen Maxi Prep
- Spin cells 4°C 4500 rpm 15 min
Pour off supernatant
Resuspend pellet in 10 ml P1 + RNase
Add 10 ml P2 mix well
Let sit RT 5 min
Add 10 ml cold P3 mix
well
Let sit on ice 20 min
Spin 4500 rpm 15 min
Equilibrate Tip 500 with 10 ml QBT
Apply Supernatant through pin wipe
Wash Column 2x
with 30 ml QC Buffer
Elute DNA @ 15 ml QF
Add Isopropanol 0.7 Volumes (10.5 ml)
Mix Well
Spin 9K GSA Rotor 30 min
Pour off Supernatant &
Add 10/5 ml 70% Ethanol, (ice
cold)
Spin 15 min 9K GSA Rotor

Maxi Prep

HTPAN0SS04 - pA2 Maxi Prep

88

9/15/94

Carefully Pour off Supernatant
Let pellet to dry at
Room Temp ~ 30 min

Resuspend pellet in 400 ul of TE
Transfer to 2ml Tube
Read OD 260/280
Dilute 1:200
DNA 5 ul → 995 ul H_2O.

Sample ID	abs 260.0 nm	abs 280.0 nm	bkg abs 320.0 nm	260.0 nm / 280.0 nm	280.0 nm / 260.0 nm		
1 pA1	0.1179	0.0760	0.0188	1.7324	0.5772	1.18 ug/ul	472 ug
2 pA2	0.1309	0.0847	0.0170	1.6834	0.5940	1.31 ug/ul	524 ug

Store 4°C in Plasmid Box.
Will need to do IP - Transfections

Run 0.5 ul of Plasmid + pA2 and
Lambda Marker

too much λ marker, but
clones look good

9/22/94

Gave Jian Ni
20 ul of each plasmid for him
to transfect COS Cells.
as per Steve Ruben's Request (9/8/94)
(pA III)

HEZ0142

Plate 50 µl on LB + Amp + IPTG/X-gal plate 9/26/94

9/27/94

Plates did Not grow.
Try PCRing the lesions.

① M13R + FP10 ~500 bp
② RP01 + FP09 ~ 430 bp.
③ M13R + FP08 ~ 1040 bp

		27x
M13R	0.1 µl	2.7
FP10	1.3	35.1
10x dNTP	3.5	94.5
10x PCR	3.5	94.5
Taq	0.2	5.4
H_2O	21.4	577.8
Rescue	5	

		27x
M13R	0.1	2.7
FP08	1.1	29.7
	3.5	94.5
	3.5	94.5
	0.2	5.4
	21.6	583.2
	5	

		3	27x
RP01	0.2	5.4	
FP09	1.3	35.1	
	3.5	94.5	
	3.5	94.5	
	0.2	5.4	
	21.3	575.1	
	5		

⊕ Control HEZ0142 plasmid
⊖ Control H_2O

Run PCR prog #69

95°C 5 min
95°C 20 sec
55°C 20 sec } 30x
72°C 1 min
72°C 7½ min
4°C Hold.

Run Pic on gel as - forgot to add
Run 1 Kb ladder

Aleem Rk
9/27/94

HTPB411515 & HTPAN08504 into PDYO

Pg 83

9/28/04

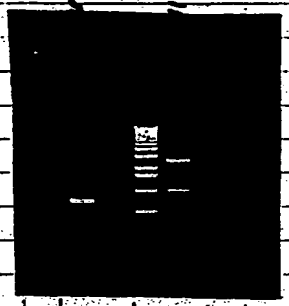
Fragment prep for HTPB411515 3'Xba 5'Bam
HTPAN08504 3'Xba 5'Bam

HTPB411515		HTPAN08504	
DNA	1	DNA	1
10xdNTP	10	10xdNTP	10
10x PCR	10	10x PCR	10
2501	0.3	2499	0.3
2502	0.3	2500	0.3
Taq	0.3	Taq	0.3
H_2O.	78.1	H_2O	78.1
	100 µl.		100 µl

Do 10 Rxns of each and 1 negative control.

PCR Prog #69
95°C 5 min
95°C 20 sec
55°C 20 sec } 30X
72°C 1 min.
72°C 7 1/2 min.
4°C hold.

Run 5ul on gel with 1 kb ladder



- HTPAN08504 ~ 800 bp
- HTPB411515 ~ 2700 bp.

- PEG precipitate - equal Volume
Spin 10 min
Remove supernatant
Add 1 ml 70% Ethanol
Spin 5 min
Remove Supernatant

Allow pellet to dry
Resuspend in 50 µl of TE

 HJPB411SO4 & HTPAN08SO4 in pDIO

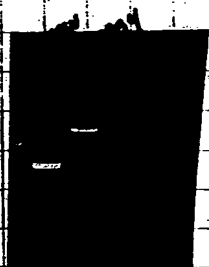
9/28/94

Run on 0.80% LMP gel

Cut out of gel
Gene Clean
Add 1ml NaI
Heat 55°C 5 min
Mix
Add 7ul Glass Milk
Let sit at RT 2min
with occasional mixing
Spin 5 sec
Remove supernatant
3X [Resuspend pellet in 400ul Wash Buffer
Spin 5 sec
Remove supernatant]

Spin 5sec
Remove supernatant
Add 20ul TE
Heat 55°C 2 min
Spin 5sec
Transfer to fresh tubes
Add 20ul TE
Heat 55°C 2 min
Spin 5sec
Transfer to tube
- Run 2ul on gel with 1Kb ladder.

9/29/94



Looks good
Digest with Xba + Bam

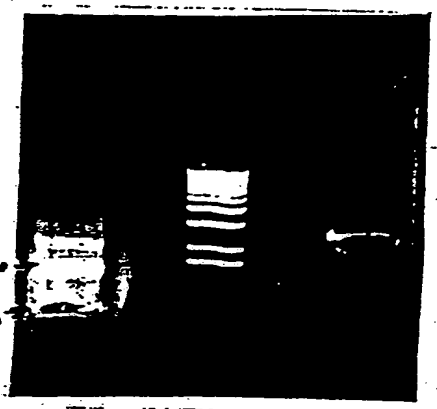
DNA	30ul
10x#2	5
H_2O	13
Enzyme	1
	50ul

Digest with Bam for 4 hrs at 37
Then add 1ul Xba & digest at 37°C for 4 more hours.
Store 4°C

HTPB4/1515 + HTPAN08504 in PDTO 113

9/30/94

Run samples on 0.8% LMPgel



HTPAN08504 Bam/Xba
Digest
Cut out bnds 0.9 Kb
and 1.6Kb fragments
HTPB4/01515 Bam/Xba
Digest
Cut out 2.4Kb frags

Gene Clean
Add 1ml NaI
Heat 55C 5min
Add 7ul Glass milk
mix & let sit at RT 2min

Spin 5sec
Remove supernatant
5X { Resuspend pellet 400µl Wash Buffer
Spin 5sec
Remove supernatant }
Spin 5sec
Remove supernatant
Resuspend pellet 20µl TE
Heat 55°C 2min
Spin 5sec
Transfer to fresh Tube
Add Resuspend pellet in 20ulTE
Heat 55°C 2min
Spin 5sec
Transfer to tube
Run 5ul on gel with 1Kb ladder

1-0.9Kb HTPAN08504
2-1.6Kb HTPAN08504
3-2.4Kb HTPB4/1515

HTPAN08504 & HTPB411S15 in PD10

9/30/94

Set up ligations.

① HTPAN08504 0.9Kb Bam/Xba
② HTPAN08504 1.6Kb Bam/Xba
③ HTPB411S15 2.4 Kb Bam/Xba

DNA Fragment + Vector.

#1	①	4ul +	pD10	Bam/Xba	2ul
#2	①	4ul +	pD10	Xba/Bam	2ul
#3	②	6ul +	pD10	Bam/Xba	2ul
#4	②	6ul +	pD10	Xba/Bam	2ul
#5	③	4ul +	pD10	Bam/Xba	2ul
#6	③	4ul +	pD10	Xba/Bam	2ul.
#7	①	4ul	+	———	
#8	②	6ul	+	———	
#9	③	4ul	+	———	
#10		———	+	pD10	Bam/Xba
#11		———	+	pD10	Xba/Bam
#12		———	+	pBSK	cut digest
#13		———			

2ul Total Rxn Volume
⊖ control w/5 cells only ⊕ control long pD10
Incubate RT 1 hr
Add 10 ul to 100 ul MM5 cells
Let sit on ice 1 hr
Heat 42°C 45 sec
Sit on ice
Add 400 ul LB
Let shake at 37°C 1 hr
plate 200 ul on LB + Amp + Kan plates for pD10 vectors
plate 100 ul on LB + Amp + B gal plate for pBSK.
Let sit RT over the weekend.

HTPAD08504 & HTPB411515 in PD10

10/3/94

Picked ⑥
Cgnes of HTPAD08504 &
HTPB411515
into 200 µl LB + Amp + Kan.
DNA Incubate 37°C; 4hrs.
Do PCR.

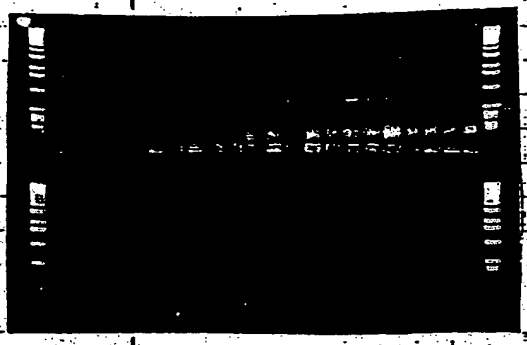
HTPAD08504		HTPB411515	
10x PCR	3.2	10x PCR	3.2
10x dNTP	3.2	10x dNTP	3.2
2499	0.3	2501	0.3
2500	0.3	2502	0.3
Taq	0.2	Taq	0.2
H_2O	22.8	H_2O	22.8
Culture	2 µl	Cult	2
	32 µl		32 µl

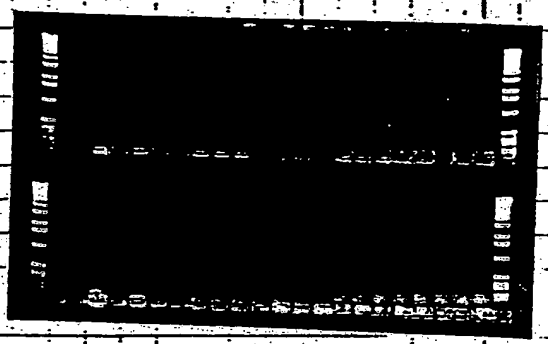
PCR Program # 69.

95°C	5 min	
95°C	20 sec	30x
55°C	20 sec	
72°C	1 min	
72°C	7½ min	
4°C	Hold	

Run 10 µl on gel with 1Kb ladder

⑥ controls -
use 10ng Plasmid
DNA

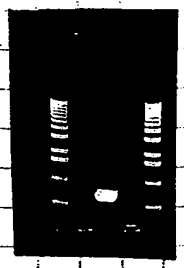




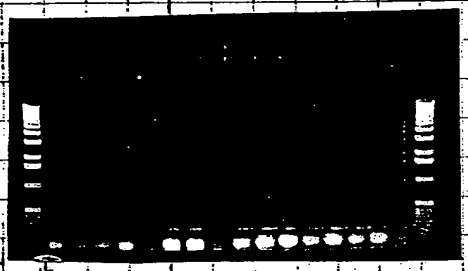
HTPAD08

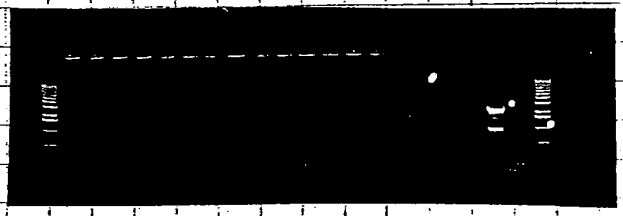
HTPA308 & HTPB411 S15 on PD10

10/3/94



HTPA308





HTPB411

Transform Remaining Amount of Ligation into M15 cells

Add DNA to 100µl M15 cells
Incubate 1 hr.
Heat 42°C 45sec
Place on ice
Add 400µl LB
Incubate 1 hr.
Plate onto LB + Amp + Kan Plates
Incubate O/N at 37°C

10/4/94

Pick Colonies and Into LB + Amp + Kan media in 96 well dish.
Incubate 37°C w/ aeration

Rx PCR

See Plan Map from 115

See Pg 123

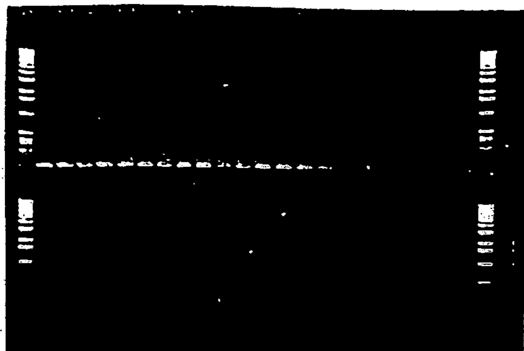
HTPAN08 ∘ H1PB44 in PD10

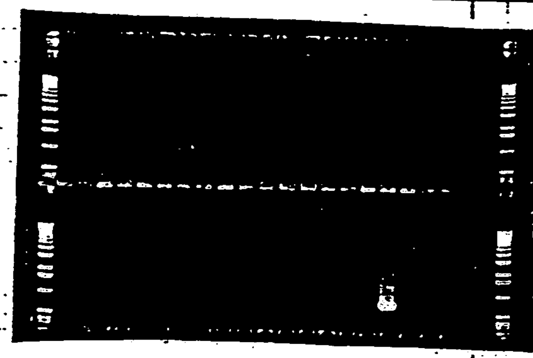
(pg 116)

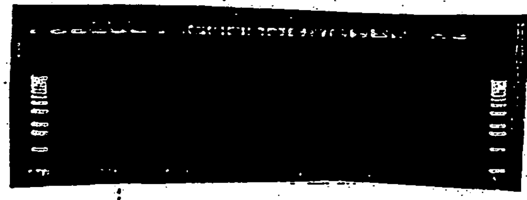
10/4/94

PCR Prog #69.

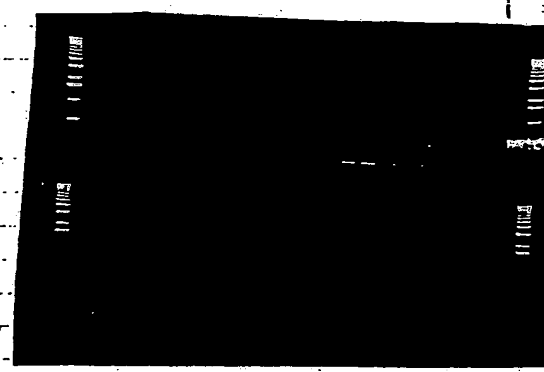
Run 10ul on Gel w/ 1kb ladder

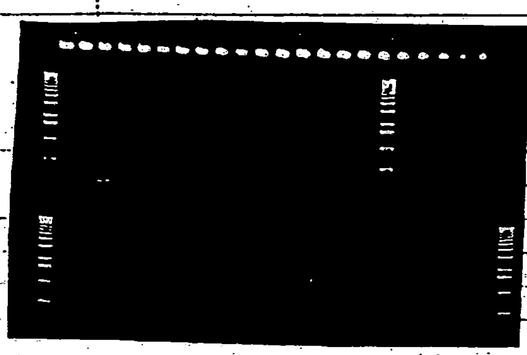






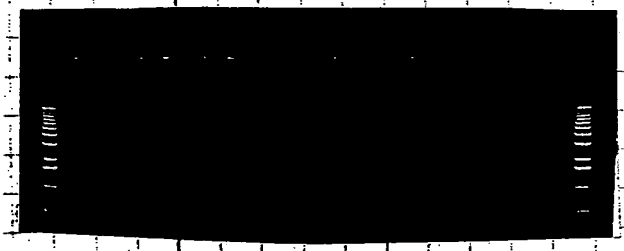
HTPAN08

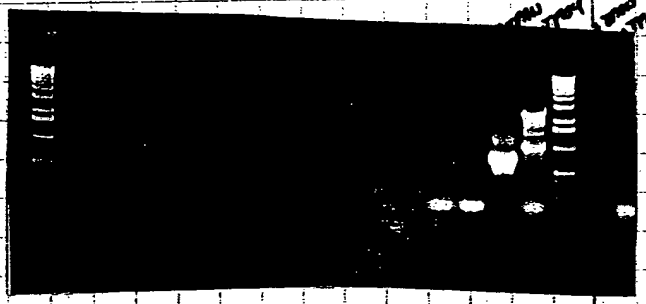




124 HTPAN08 & HTPB411 in pDTO

10/4/94





Looks like 10 for HTPAN08.
Remake 10 pDTO Bam/Xba fragVector.
Grow up Maxi Prep.

10/5/94

Re set up digations

	1	2	3	4	5	
HTPAN08 0.9	6	—	—	—	—	
HTPAN08 1.6	—	8	—	—	—	
HTPB411 SIS	—	—	8	—	—	
PD10 Bam/Xba	2	2	2	2	—	
10X	2	2	2	2	2	
T4 Ligase	1	1	1	1	1	
H2O.	9	7	7	15	17	

Incubate 16°C O/N.

10/6/94

Transform to MIS cells

10/6/94

to 100 µl M15 Chemically Competent Cells add 10 µl of ligation
Incubate all on ice 1 hr
Heat 42°C 45 sec
Place on ice
Add 400 µl LB
Incubate 37°C 1 hr.
Plate 200 µl onto LB + Amp + Kan 150mm plates
Incubate 37°C O/N.

10/7/94

~~[illegible]~~
Colonies did not grow well
only about <10 colonies per plate.

Remake pD10 vector.
Remake insert fragments

10/10/94

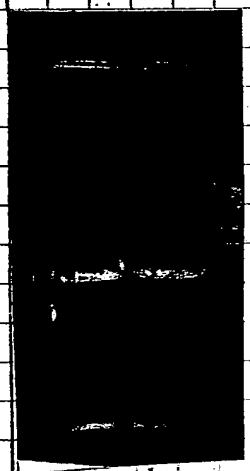
Digest PD10 1.57 ug/µl

DNA	3.2
10X	10
H_2O	84.8
Bam/Xba	1/1
	100 µl.

Incubate 37°C O/N

10/11/94

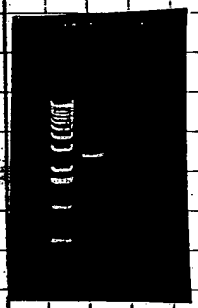
Run on 0.8% LMP Gel with 1Kb ladder
80V 1½ hrs.



Isolate gel fragment cuts ~ Tube 1-20
Add 1000 µl NaI.
Heat @ 55°C 5 min until melted
Mix well
Add @ 7 µl Glass milk
Mix well
Incubate at RT 5 min with occasional mixing
Spin 7 sec
Remove supernatant
Resuspend pellet in 400 µl Wash Buffer.

3X
Spin 7 sec
Remove Supernatant
Spin 7 sec
Remove supernatant
Resuspend pellet 20 µl TE
Heat @ 65°C 1 min
2X
Spin 10 sec
Transfer & Combine cuts 1 fresh tube

Run 2 µl on gel with 1Kb ladder.



Make more fragment
(See pg 9.40)

Hr RCC91 HPRDM93

10/13/94

Develop film.

Pick ⊕ Clones into 50ul SM Buffer.
Only HPRCC91 seemed to work —
115 ⊕ clones
HPRDM93 - Nothing Let up.
Try HPRDM Again 0 — (See pg 141)
2000

10/18/94

Do PCR on ⊕ clones from 1° phage.

		120x
10x PCR	3.5	420
10x dNTP	3.5	420
M13R	0.1	12
(5235) FR03	2	240
Taq	0.2	24
H_2O	20.7	2484
phage	5	
	35ul	30ul/tube

Run PCR Program #69.

95°C 5min
95°C 20 sec
55°C 20 sec } 30X
72°C 1min
72°C 7½ min
4°C Hold

Run 10µl on 1% Aspire gel with 1kb ladder

Use HPRCC93 plasmid DNA as ⊕ Control
Use H_2O as ⊖ Control.

Nan Thele
10/15/94

146 HTPB4II & HTPAX08 into PD10

pg 126

10/11/94

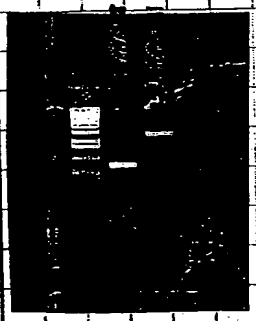
Take PCR'd product from.
(9/28/94 pg 112)

Digest

DNA	3
10x Buffer	5
H2O	41.0
Xba/Bam	0.5/0.5
	50µl

Incubate 37°C 4hrs

Run on 0.8% LMP gel
Excise Band
Gene Clean (See pg 128 for protocol)
Run 2µl on gel



10/12/94

Set up ligations again.

With PD10 from 10/11 &
HTPAX08 band from ~~10/11~~ HTPB4II Bam/Kpn from 10/11

HTPB411 & HTPAN08 cuts PD10 147

10/12/94

[illegible]

	1	2	3	4	5	6	7	8
HTPAN0809	4	—	—	4	—	—	—	—
HTPAN0816	—	4	—	—	4	—	—	—
HTPB411	—	—	4	—	—	4	—	—
PD10 B/xba	1.5	1.5	1.5	—	—	—	1.5	—
10X T4 Buffer	2	2	2	2	2	2	2	2
H_2O	11.5	11.5	11.5	13	13	13	13.5	17
T4 ligase	1	1	1	1	1	1	1	1
	20	20	20	20	20	20	20	20

Incubate 16°C O/N.

10/13/94

Transfor M15 cells.

Thaw M15 Chem. Competent cells on ice

To sterile tubes Combine
100 µl M15 cells
10 µl of ligation
} use 10ng PD10 as @ control.

Let sit at RT 1 hr
Heat 42°C 45 sec
Quick Chill
Add 400 µl LB
Incubate 37°C 1 hr
plate 200 µl onto LB + Ampt + Kan plates (150mm)
Incubate 37°C O/N

148 HTPBY11 & HTPAN08 with PDI0

10/14/94

Plates look OK -
Plates with pDI0 Vector alone has many colonies
Pick 100 of each Clone into 200 ml of 0 LB + Ampicillin
Incubate at 37°C w/ aeration

PCR.

HTPA008		HTPBY11	
2499	0.2	2501	0.2
2500	0.2	2502	0.2
10x dNTP	3.2	10x PCR	3.2
10x PCR	3.2	10x dNTP	3.2
Taq	0.2	Taq	0.2
H2O	23	H2O	23
Culture	2	Culture	2
	32 µl		32 µl

Perm Program #69.

95°C	5min	
95°C	20sec	30x
55°C	20sec	30x
72°C	1min	30x
92°C	7 1/2 min	

Run 10µl on gel with 1Kb ladder -
Nothing Showed up!

Try Again

HTPB411 & HTPAN08 into PD10

10/19/94

PCR Amplify fragments Again

① HTPAN08

2499	0.2
2500	0.2
10x PCR	10.0
10x dNTP	10.0
Taq	0.3
DNA template	1 (10ng/µl)
H2O	78.3
	100µl

② HTPB411

2501	0.2
2502	0.2
10x PCR	10
10x dNTP	10
H2O	78.3
Taq	0.2
DNA	1
	100µl

Set up 5 tubes of each
Run PCR.

95°C	5min	
95°C	20sec	30x
55°C	20sec	
72°C	1min	
72°C	7½ min	

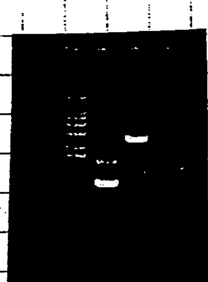
Run 10 µl on gel with 1kb ladder

Precipitate PCR Prep
Add equal Volume
13% PEG / 1.6M NaCl
Let Set on ice 15 min
Spin 15 min
1x 70% ethanol wash
Dry pellet slightly
Resuspend in 50µl TE

HTPBY11 & HTPAN08 into pD10

10/19/94

Run Gel on 1% TAE gel with 1 Kb ladder



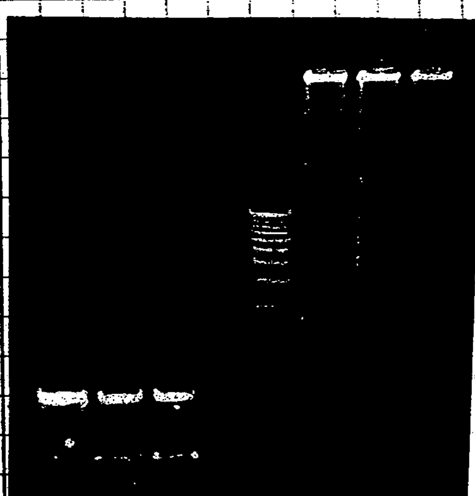
looks good —
Set up Digestions

DNA	40ul
H_2O	48ul.
10X #2	10
Xba/Bam	1/1
	100ul

Incubate 37°C O/N.

10/20/94

Run on 0.8% LMP Gel with 1Kb ladder.



Gene Clean
See Page 126

Set up ligations
with pD10 from 10/19 - See pg 151

See pg 3 book 8